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Glucose deprivation mediates interaction between CTDK-I and Snf1 in *Saccharomyces cerevisiae*

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Abstract Ctk1 is a kinase involved in transcriptional control. We show in the two-hybrid system that Ctk1 interacts with Snf1, a kinase regulating glucose-dependent genes. Co-purification experiments confirmed the two-hybrid interaction but only when cells were grown at low glucose concentrations. Deletion of Ctk1 or its associated partners, Ctk2 and Ctk3, conferred synthetic lethality with null mutants of Snf1 or Snf1-associated proteins. Northern blot analysis suggested that Ctk1 and Snf1 act together in vivo to regulate *GSY2*. These findings support the view that Ctk1 interacts with Snf1 in a functional module involved in the cellular response to glucose limitation.

Keywords: Ctk1; Snf1; Interaction; Diauxic shift; Yeast

1. Introduction

Ctk1, with the cyclin Ctk2 [1] and the co-cyclin Ctk3 [2], is part of the non-essential CTD kinase complex I (CTDK-I) complex that was first isolated for its ability to phosphorylate in vitro a purified recombinant form of the carboxy terminal domain (CTD) of RNA Polymerase II (Pol II) [3]. Much evidence supports its role in the control of transcription elongation: (i) CTDK-I promotes efficient elongation in HeLa nuclear extracts through its kinase activity [4] and affects Pol II processivity [5], (ii) the phosphorylation of serine 2 of the CTD during elongation is largely dependent on Ctk1 [6], (iii) *ctk1*Δ and *ctk3*Δ mutants are sensitive to 6-azauracil and mycophenolate nucleotide-depleting drugs thought to impair elongation [7,8], and (iv) *ctk1* null mutants exhibit synthetic lethality or synergy with defective alleles of various genes known to play a role in elongation (e.g., *dst1*Δ) [8,9]. In addition to its role in transcriptional elongation, Ctk1 has also been implicated in mRNA maturation, including splicing [10,11] and 3′ end formation [12,13], in mRNA nuclear export [14], in histone methylation [15], in DNA damage-induced transcription [16], in the response to nutrient depletion [17] and in glucose repression/derepression [18]. Interestingly, a recent work suggested that Ctk1 plays a role in Pol I transcription and that Ctk1 might coordinate the Pol I and Pol II machineries [19].

In this paper, we report the results of a two-hybrid screen using Ctk1 as a bait that identified the Snf1 kinase as a physical partner. The in vivo interaction seems dependent on nutritional conditions since co-purification of Ctk1 and Snf1 could only be observed at low glucose concentrations. The CTDK-I and Snf1 complexes were consistently linked functionally and acted together to induce *GSY2* transcription during the diauxic shift. Our data suggest that CTDK-I plays a specific role in glucose-dependent transcriptional regulation in yeast.

2. Materials and methods

2.1. Yeast strains and plasmids

The *S. cerevisiae* strains Y06774 (*ctk2*Δ), Y06512 (*ctk3*Δ) and Y04311 (*snf1*Δ) were derived from BY4741 (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*); Y14311 (*snf1*Δ) was derived from BY4742 (*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*) and Y24989 (*CTK1/ctk1*Δ) was derived from BY4743 (*MATa/MATαhis3Δ1/his3Δ1 leu2Δ0/leu2Δ0 lys2Δ0/LYS2 MET15/met15Δ0 ura3Δ0/ura3Δ0*). All these mutant strains contain full deletions with a *kanMX4* insertion and are from Euroscarf (http://www.uni-frankfurt.de/fb15/mikro/euroscarf/). YVB08-4C (*MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*) and YVB06-4A (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ctk1::kanMX4*) are, respectively, a wild type and a *ctk1*Δ offspring of Y24989. YC176-1D (*MATα his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ctk1::kanMX4 snf1::kanMX4*) was obtained through a meiotic cross between YVB06-6A and Y14311. YVB118 (*MATa ade2-101 lys2-801 ura3-52 trp1-Δ63 his3-Δ200 leu2-Δ1 SNF1::3HA::KanMX6*) is a mutant derived from YPH499 [20] with a haemagglutinin tag fused to the C-terminal end of Snf1 constructed as described in [21].

The Y190 strain (*MATa gal4Δ gal80Δ his3Δ200 trp1-901 ade2-101 ura3-52 leu2-3,-112 CYH1^R URA3::GAL1::LacZ LYS2::GAL4(UAS)::HIS3*) [22] was used for the two-hybrid tests. Over 10^7 transformants were screened on medium lacking the appropriate amino acids and supplemented with 100 mM 3-amino-1,2,4-triazole (Sigma). The resulting colonies were then tested for β-galactosidase activity in an overlay assay [23] and colonies that activated the two reporter genes were selected. Sequencing of the library plasmids in these colonies led to the identification of 6 partners: Gbp2, Pep8, Prs1, Rgd1, Snf1 and Sqt1.

The plasmids used are listed in Table 1. pVB06 was constructed through *Sma*I/*Sal*I directional cloning of a polymerase chain reaction (PCR)-amplified *CTK1* insert into pGBT9. The construct was verified by DNA sequencing. pSNF1-177 was isolated from the two-hybrid library. pVB20 and pVB21 were constructed using the GATEWAY™ technology [24,25] as previously described [26]. For pVB20, the pACTII was first adapted to the GATEWAY™ technology by inserting the so-called Gateway™ B cassette (recovered by *Eco*RV digestion of the pSK-B plasmid [26]) at the *Sma*I site of pACTII to yield the pVV213 vector.

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Abbreviations: 3AT, 3-amino-1,2,4-triazole; CTD, carboxy terminal domain; CTDK-I, CTD kinase complex I; PCR, polymerase chain reaction; $Gal4_{AD}$, Gal4 activation domain; $Gal4_{DB}$, Gal4 binding domain; Pol I, II; RNA polymerase I, II, respectively; P-TEFb, positive transcription elongation factor b; TREX, transport/export complex

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Table 1
Plasmids used in this study

Name	Yeast genes	Backbone vector
pVB06	2μ TRP1 $Gal4_{DB}$(1–147)::CTK1	pGBT9 [58]
pVB20	2μ LEU2 $Gal4_{AD}$(768–881)::SNF1,1-633	pACTII [31]
pVB21	CEN URA3 $ptetO_7$::CTK1::13Myc	pVV211 [26]
pSNF1-177	2μ LEU2 $Gal4_{AD}$(768-881)::snf1,95-392	pACTII

2.2. Growth conditions

The yeast cultures were grown in YPD medium (1% yeast extract, 2% bactopeptone, 2% dextrose) or a synthetic medium lacking the appropriate amino acids. The carbon-source concentrations were as follows: 2% glucose and galactose and 3% raffinose, glycerol and ethanol. When a shift was used, the yeast cells were first grown in 2% glucose to mid-log phase. After one wash with water, the culture was split in three and shifted in fresh 2% glucose, 0.05% glucose or 2% galactose media. After 2 h at 30 °C, the cells were collected by filtration and frozen by immersion in liquid nitrogen.

2.3. Measurement of β-galactosidase activity

The yeast cells were grown in a synthetic 2% glucose medium as described previously [27] and β-galactosidase activity was measured from permeabilized cells [28].

2.4. Protein extraction and purification

The proteins were extracted by glass bead lysis in ST buffer (50 mM Tris–HCl, pH 8.0, 150 mM NaCl) supplemented with 2 mM phenylmethylsulfonyl fluoride (see [29]). 500 μg proteins were incubated with 150 μl of Ni^{2+} charged Hisbind® resin (Novagen) in a final volume of 250 μl for 2 h on a rotating wheel at 4 °C. The beads were pelleted by quick centrifugation and washed with 1 ml of cold washing buffer (ST buffer with 1% Triton X-100, 0.05% ovalbumin and 52.5 mM imidazole). This wash was repeated 13 times and was followed by a final wash with a washing buffer containing 55 mM imidazole. The bound proteins were eluted with 100 μl of ST buffer containing 0.2 M EDTA, heated for 5 min at 100 °C, separated by SDS–PAGE and revealed using monoclonal anti-HA or anti-Myc antibodies (Babco) with the ECL™ Western Blotting Detection kit (Amersham Biosciences).

2.5. RNA and Northern blot analysis

Total RNA was isolated from yeast cells using hot phenol [30]. 30 μg were separated by electrophoresis on a 1.2% agarose–formaldehyde gel and transferred to a nylon membrane. The RNA were detected by PCR-amplified DNA fragments of the target gene as probes labelled with ^{32}P using the Random Prime labelling kit (Invitrogen).

3. Results

3.1. Ctk1 interacts physically with the N-terminal domain of Snf1

We carried out a two-hybrid screen using a $Gal4_{DB}$ ($Gal4_{DB}$) (1–147)::Ctk1 fusion as a bait and a random library of yeast genomic fragments fused to the Gal4 activation domain ($Gal4_{AD}$) (768–881) [23,31]. In a preliminary step, we showed that the $Gal4_{DB}$ (1–147)::Ctk1 fusion construct was able to complement the cryosensitive phenotype of a *ctk1*Δ mutant (data not shown). Out of more than 10^7 transformants, 12 clones activated the expression of the two reporter genes (*HIS3* and *lacZ*), among which 4 corresponded to two nuclear proteins: Gbp2 (2 clones) and Snf1 (2 clones) (Fig. 1A and data not shown). The localisation of these two proteins in the nucleus, the known location of Ctk1 [19,32], brought these two candidates to our attention.

GBP2 is a poly(A)$^+$ RNA-binding protein associated with the transcription/export complex (TREX) [14,33]. Our two-hybrid data corroborate the presence of interaction between Gbp2 and Ctk1 previously reported in the results of a TAP-tag purification [14,34].

The second partner, Snf1, is the central kinase required for growth on alternative sugars and non-fermentable carbon sources [35–37]. The two clones corresponding to Snf1 encoded for an identical fragment spanning amino acids 95–392 (Fig. 1B). Snf1 is comprised of a catalytic domain (residues 1–392) and a regulatory domain (residues 393–633) that inhibits Snf1 activity by binding to the catalytic domain [38]. Interestingly, the fragment isolated in the screen includes most of the N-terminal domain of Snf1 without the regulatory domain. In a quantitative β-galactosidase assay, the two-hybrid interaction was observed with the Snf1 fragment lacking the regulatory domain but not with full length Snf1 (Fig. 1C), suggesting that the regulatory domain not only inhibits Snf1 kinase activity but may also impede interaction with Ctk1.

3.2. Ctk1 co-purifies with Snf1 at low glucose concentrations

The two-hybrid results suggest that Snf1 could be a physical partner of Ctk1 in vivo. To confirm this data, we examined whether the purification of Snf1 from cell-free extracts leads to the co-purification of Ctk1. Using the natural His tag in the N-terminal region of Snf1 (residues 13–30), we purified Snf1 from a *SNF1::3HA* or *snf1*Δ strain expressing Ctk1::13Myc. At high glucose concentrations (2%, glucose-repressed), no interaction between Snf1 and Ctk1 was observed (Fig. 2A, first two lanes). Given that Snf1 undergoes changes in conformation and subcellular localisation as a function of the carbon source used [39] and that Ctk1-dependent CTD phosphorylation is known to occur during the diauxic shift [17], we tested whether the carbon source influences the interaction between Snf1 and Ctk1. As shown in Fig. 2B, Snf1 only pulled down a significant amount of Ctk1::13Myc when cells were grown in low glucose concentrations (0.05%, probably mimicking diauxic shift conditions). Co-purification was, however, not detected when cells were grown in 2% galactose (glucose-derepressed). These two-hybrid and co-purification results, and especially the observation that Ctk1 is only co-purified with Snf1 in media depleted in glucose, strongly suggest that the interaction is a physiological component of the adaptation to glucose limiting stress.

3.3. CTDK-I mutants display growth defects on some non-glucose media

A great amount of data has already demonstrated the key role of Snf1 in carbohydrate metabolism [35–37]. Ctk1, on the other hand, was also shown to be involved in carbohydrate metabolism on the basis of a slow growth phenotype of a *ctk1*Δ strain on galactose and glycerol media [18]. We further demonstrate that mutants of each single Ctk subunit of CTDK-I display slow growth on galactose, glycerol and to some extent on ethanol but not on raffinose (Fig. 3). Thus, our data confirm the role of CTDK-I in the adaptation of yeast to alternative carbon sources and further suggest that each of the three Ctk subunits of the complex contribute to this

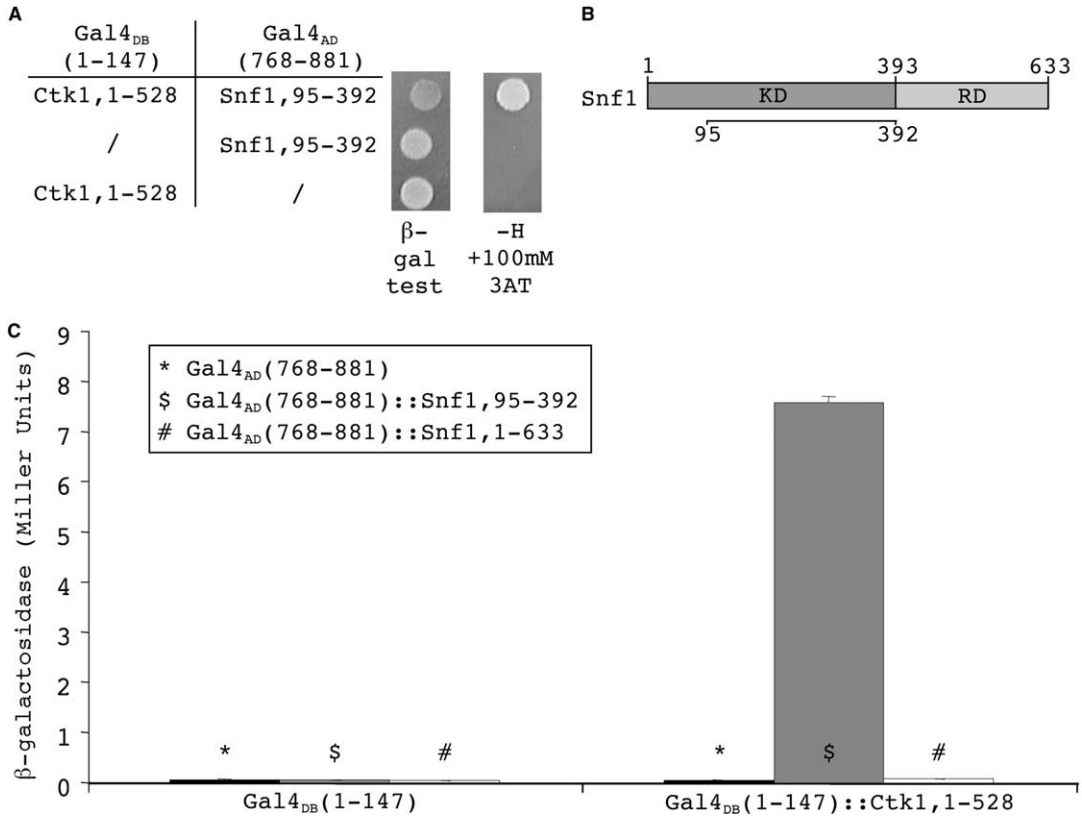


Fig. 1. Two-hybrid interaction between Ctk1 and Snf1. (A) Two-hybrid interaction between Ctk1 and Snf1. The coding sequence of Ctk1 was fused to the $Gal4_{DB}$ (1–147) (plasmid pVB06, see Table 1) and tested for interaction with a fragment of Snf1 (residues 95–392, plasmid pSNF1–177). Both plasmids were transformed in strain Y190 and tested for β-galactosidase activity in an overlay assay (left panel) or for growth on a medium without histidin but containing 100 mM 3AT (right panel). (B) Schematic representation of Snf1. The two domains are shown as described by Ludin and co-workers [38]. KD: kinase domain (this kinase domain can be restricted to residues 55–306 using the pfam software, http://pfam.wustl.edu/); RD: regulatory domain. The thick horizontal line corresponds to the fragment isolated in the two-hybrid screen. (C) Liquid β-galactosidase activity assay. The means and standard deviations were calculated from assays on three independent clones. The plasmids used were identical to those described in (A) except that a full-length Snf1 fused to the $GalA_{AD}$ (768–881) was also used (pVB20).

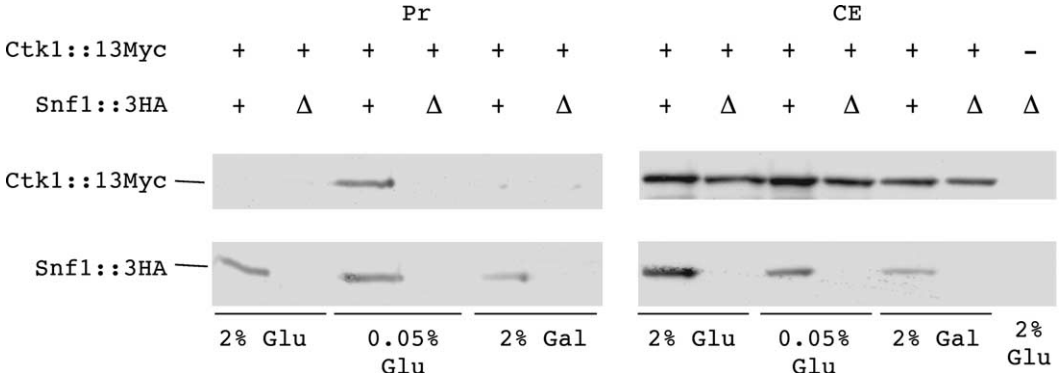


Fig. 2. Co-purification of Ctk1 with Snf1 in cell-free extracts. Strains YVB118 (*SNF1::3HA*) and Y14311 (*snf1*Δ) were transformed with a centromeric plasmid encoding the Ctk1::13Myc fusion (pVB21). The resulting strains were grown to mid-log phase and then induced for 2 h in fresh medium with the carbon source indicated. Snf1 was precipitated from the crude extract using its natural His tag and the precipitated proteins (Pr) were separated by SDS–PAGE together with 50 μg of crude extract (CE). The presence of Ctk1::13Myc (upper panel) and Snf1::3HA (lower panel) was revealed by Western blotting using anti-Myc or anti-HA antibodies, respectively.

function. Since mutants of the CDK8/Srb10/Ssn3 CTD kinases do not display similar phenotypes on the same media (data not shown), we conclude that the delayed growth of the three CTDK-I mutants on alternative carbon sources is a specific effect.

3.4. The CTDK-I and Snf1 complexes show functional overlap

To further substantiate the relative importance of Snf1 and Ctk1 and their interaction in the phenotype considered, we compared the double *ctk1*Δ *snf1*Δ mutant with the parental strains. Fig. 4A presents typical tetra-type segregation from

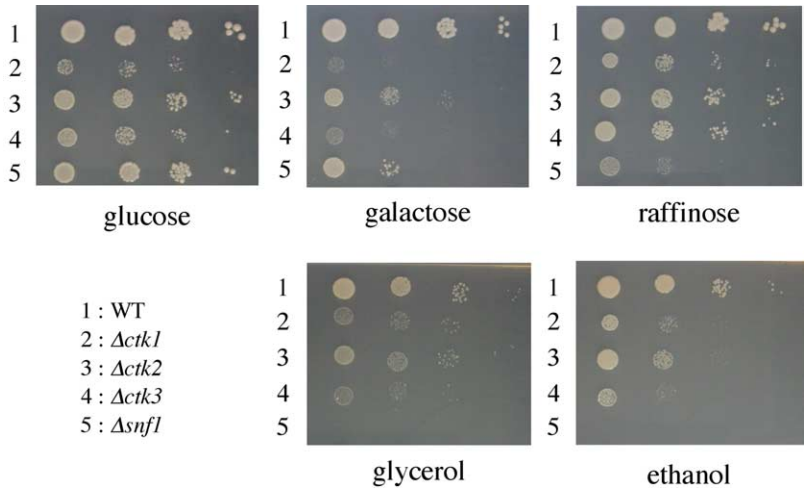
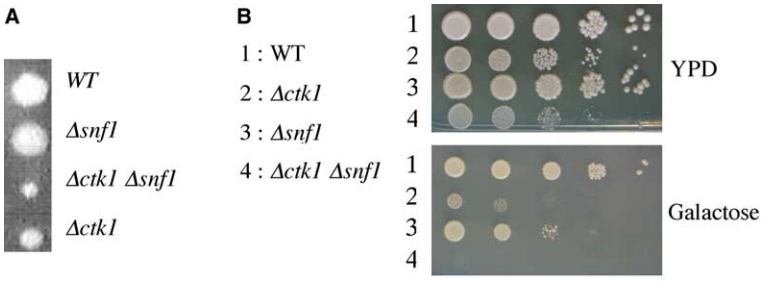


Fig. 3. Phenotypic effects of the CTDK-I mutants. Wild type (YVB08-4C), *ctk1*Δ (YVB06-4A), *ctk2*Δ (Y06774), *ctk3*Δ (Y06512) and *snf1*Δ (Y04311) were grown to mid-log phase and diluted to an OD_{600} of 0.3. Serial 10-fold dilutions were spotted on medium containing the carbon source indicated. The pictures were taken after 3 days (glucose, galactose and raffinose) or 7 days (glycerol and ethanol) of growth at 30 °C.



C

	WT	*Δsnf1*	*Δsnf4*	*Δsip1*	*Δsip2*	*Δgal83*	*Δsip1 Δsip2 Δgal83*
WT	+	+	+	+	+	+	+
Δctk1	+	-	-	+	+	+	-
Δctk2	+	-	-	+	+	+	-
Δctk3	+	-	-	+	+	+	-

Fig. 4. CTDK-I and Snf1 complexes are genetically linked. (A) Synergy between *ctk1*Δ and *snf1*Δ. The *ctk1*Δ strain (YVB06-4A) was crossed with a *snf1*Δ mutant (Y14311). The offspring tetrads were grown on YPD for 5 days at 30 °C before the picture was taken. Typical tetra-type segregation is shown. (B) Synergy between *ctk1*Δ and *snf1*Δ. Wild type (YVB08-4C), *ctk1*Δ (YVB06-4A), *snf1*Δ (Y14311) and *ctk1*Δ *snf1*Δ (YC176-1D) were spotted as described in Fig. 3 on YPD (upper panel) or minimal medium containing 2% galactose (lower panel). The plates were incubated for 3 days at 30 °C. (C) Summary of synthetic phenotypes involving CTDK-I and the Snf1 complex. The "+" symbol indicates no aggravation of the phenotype versus the parental strain phenotype. The "−" denotes a synergy marked by slower growth and barely detectable growth on galactose.

more than 20 tetrads analysed and shows a synergetic relationship between *ctk1*Δ and *snf1*Δ. This was also evident from the comparison of the growth rate of the strains in rich liquid medium at 30 °C. Indeed, the *ctk1*Δ *snf1*Δ mutant required 205 min to spread from an $OD_{600\,nm}$ of 0.3–0.6 whereas it took only 96, 138 and 113 min for the isogenic wild type, the *ctk1*Δ and *snf1*Δ strains, respectively. In addition to the delayed growth of the *ctk1*Δ *snf1*Δ mutant observed in YPD, growth of this double mutant was barely detectable on synthetic medium containing galactose (Fig. 4B). Complementation experiments were performed to ensure that these phenotypes were not caused by a spurious mutation. The *ctk1*Δ *snf1*Δ mutant expressing a wild type copy of *SNF1* or *CTK1* from a centromeric plasmid grew at a rate similar to the corresponding single mutant, confirming that the synergetic relationship between *ctk1*Δ and *snf1*Δ is specific to these two mutations (data not shown).

Next, we tested the functional relationship between the CTDK-I and the Snf1 complexes. The Snf1 complex is composed of one catalytic α subunit (Snf1), one of the three β subunits (Sip1, Sip2 or Gal83) and one γ subunit (Snf4) [29,40,41]. Combination of the *ctk*Δ deletants (*ctk1*Δ, *ctk2*Δ or *ctk3*Δ) with the *snf1*Δ or *snf4*Δ null mutant, but not with *sip1*Δ, *sip2*Δ or *gal83*Δ, yielded a phenotype similar to that of the *ctk1*Δ

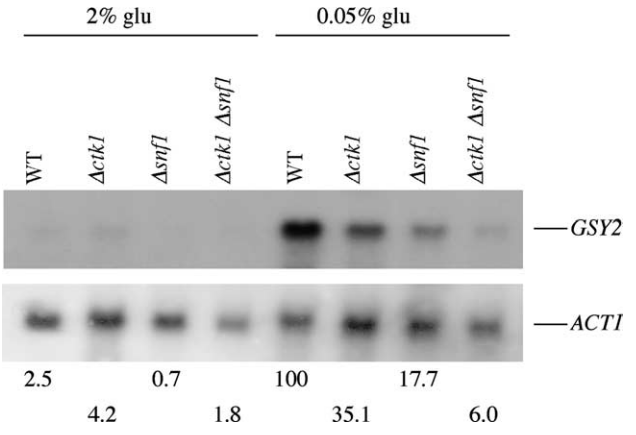


Fig. 5. Northern blot analysis of *GSY2* mRNA. Total RNA was prepared from wild type (WT), *ctk1*Δ, *snf1*Δ and *ctk1*Δ *snf1*Δ after 2 h induction at high (2%) or low (0.05%) glucose concentrations. 30 μg were then separated on an agarose gel containing formaldehyde. An autoradiograph of the membrane after hybridisation with a *GSY2* (upper panel) or an *ACT1* (lower panel, loading control) probe is shown. The *GSY2* mRNA was normalised with *ACT1* mRNA levels using the TotalLab software (Amersham Biosciences) and further compared to the wild type strain at low glucose concentrations (set at 100%). The results of this comparison are presented under the autoradiographs.

*snf1*Δ strain (Fig. 4C). As the functions of the three β subunits are related [40], we removed the three β subunits simultaneously in a *ctk*Δ background and the resulting quadruple deletants also demonstrated synergy. This further supports the hypothesis that the two complexes have overlapping functions.

3.5. The expression of GSY2 is Ctk1- and Snf1-dependent

Lastly, we examined the expression of the *GSY2* gene encoding the glycogen synthase known to be induced during the diauxic shift [42,43]. Fig. 5 illustrates that in 0.05% glucose, a condition mimicking the diauxic shift, the expression of *GSY2* was greatly reduced in the absence of Ctk1 or Snf1 (see also [17,44]). Moreover, the level of *GSY2* expression was even lower in the Δ*ctk1* Δ*snf1* mutant compared with the parental single mutant strains suggesting an in vivo cooperation between Snf1 and Ctk1 for the transcriptional regulation of at least *GSY2*.

4. Discussion

Ctk1 is involved in many steps of mRNA biogenesis including transcriptional elongation, 3′ end formation and nuclear export [4,6,7,12–14], but also in other functions such as DNA damage-induced transcription [16]. The deletion of *CTK1* results in a cryosensitive phenotype [32] and defective transcriptional induction of genes necessary for adaptation to nutrient depletion [17]. Conversely, Ctk1-dependent repression of specific genes has also been reported [17,18]. Although Ctk1 is known to be involved in multiple functions, high throughput approaches for mapping the yeast interactome have only identified a few potential partners of Ctk1 aside from CTDK-I [34,45]. We report here the results of a two-hybrid screen using Ctk1 as a bait. Two partners identified, Gbp2 and Snf1, were found to be of particular interest due to their localisation in the nucleus and their involvement in mRNA synthesis. Gbp2 has already been shown to interact with Ctk1 [14,34], but Snf1 was thus far not associated with Ctk1.

The two-hybrid interaction between Ctk1 and Snf1, at least its N-terminal region (residues 95–392), was further confirmed in co-purification experiments. Importantly, Ctk1 co-purified with Snf1 only at low glucose concentrations. This condition-specific interaction is consistent with the observation of an increase in Ctk1-dependent CTD phosphorylation during the diauxic shift [17]. Indeed, the diauxic shift mediates the activation of Snf1 and its migration to the nucleus using the Gal83 subunit [39], suggesting that Snf1 could transmit the low glucose stress signal to the Ctk1 kinase, thus activating it. The absence of co-purification in galactose, though Snf1 is known to be active, suggests the possible presence of a third partner or at least of an additional event specific to the low glucose concentration.

In addition to the physical interaction between Ctk1 and Snf1, we showed that deletion of each CTDK-I subunit causes, in addition to the well described cryosensitivity [32,46], slow growth on galactose, glycerol and somewhat on ethanol. The phenotypes of the null mutants in the CTDK-I subunits are to some extent similar to those of a *snf1*Δ strain. Indeed, deletion of *SNF1* causes growth defects on non-fermentable sugars and alternative carbon sources [35–37]. Furthermore, a functional redundancy between the CTDK-I and Snf1 complexes is suggested based on the synergy (slower growth and barely detectable growth on galactose) observed in the double *ctk1*Δ *snf1*Δ mutant. This synergy was further substantiated by the results of assays on *GSY2* expression. Interestingly, derepression of the invertase gene (*SUC2*) in low glucose medium was also shown to be dependent on Ctk1 [18], supporting the hypothesis that Ctk1 plays a role in diauxic shift.

Altogether, our data suggest that the involvement of Ctk1 in the adaptation to nutrient starvation might be mediated by its interaction with the Snf1 kinase. It would be plausible that the two kinases communicate through phosphorylation though we were unable to detect any kind of reciprocal phosphorylation of Ctk1 and Snf1 (data not shown). Although these findings do not exclude the possible role of phosphorylation, Ctk1-dependent phosphorylation of Snf1 does not appear well supported since Snf1 is known to migrate to the nucleus during starvation following phosphorylation in the cytoplasm [39]. Therefore, Snf1 arrives in the nucleus already phosphorylated. This is the result of the activity of one of the three cytoplasmic kinases of Snf1 which are known to be implicated in its subcellular localisation [47,48]. Moreover, the simultaneous deletion of the three kinases caused a *snf1*Δ-like phenotype [47]. It would be difficult to imagine that Ctk1 is a fourth kinase of Snf1, since Ctk1 is located in the nucleus [19,32] and does not belong to the same kinase family as the three kinases that phosphorylate Snf1 [49]. In turn, no evidence supports the hypothesis of Snf1-dependent phosphorylation of Ctk1. Indeed, Ctk1 belongs to the CDK family which is usually activated by phosphorylation on its T loop [50]. However, the threonine 338 of Ctk1, corresponding to the threonine of the T loop, does not match with the Snf1 substrate recognition motif [51] and Cak1 was recently identified as the Ctk1-activating kinase [52]. Snf1 also interacts physically with the CTD kinase CDK8 in a glucose-regulated manner, here again, no phosphorylation between the partners has been observed [53]. An alternative hypothesis to mutual phosphorylation

would be that Snf1 and Ctk1 phosphorylate another component of the CTDK-I or Snf1 complex (i.e., Ctk2 or Ctk3 [1,2]) or act together to phosphorylate one or several other, as yet, unidentified targets.

Interestingly, P-TEFb, the putative functional metazoan counterpart of CTDK-I, is required for rapid adaptation to heat stress by inducing HSP70 expression [54,55]. In *Drosophila melanogaster*, regulation of the HSP70 heat shock gene does not occur at initiation but during an early pause in elongation [56]. When the cells are heat induced, P-TEFb is recruited to the heat shock loci and stimulates the transcription of the corresponding genes, thereby allowing a rapid adaptation to the stress [57]. Given the adaptation required in response to glucose depletion, it is likely that, like P-TEFb, Ctk1, through its interaction with Snf1, is required in yeast for the quick expression of a subset of genes important during the shift from fermentation to respiration by releasing a paused polymerase (e.g., for the expression of *GSY2*). Such a "shortcut" would not be surprising. Indeed, one shortcut between Snf1 and an artificially recruited holoenzyme has already been reported [53].

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